# ***Experimental Report – Annex HE4***

## Part One: Crunchiness effect of two types of particles in the gum part vs. one type of particle

Aim: To investigate the effects on crunchiness of a chewing gum tablet including a tablet part and a gum part when (i) the gum part is compressed from gum base particles and tablet base particles vs. (ii) the gum part is compressed from pre-made chewing gum particles.

### *1.1 Preparation of samples*

#### Example 1

A plasticized rubber gum base formulation was made by mixing 20 wt. % synthetic elastomers (butyl rubber and polyisobutylene) and 40 wt. % resins (natural gum resins and polyvinylacetate) and 20 wt. % softening agents (wax and emulsifier) and 20 wt. % filler (talc). The above gum base ingredients were mixed on a Z-blade mixer at 120 °C for 120 minutes to yield a total of 400g of a gum base formulation.

After preparation of the gum base formulation, the gum base thus produced was cooled to a temperature of minus 75 °C. Then a grinding procedure performed on a Baumeister mill set to produce particles of size 0-1000 μm.

Sorbitol particles (obtained from Roquette; particle size Min 90% > 40μm, Max 5% > 600μm) were added to 360g of the ground gum base particles in a blending step in an amount of 440g.

The resulting blend was sieved to ensure a particle size between 10 microns and 2000 microns. All of the particles were found to pass through a 1500 μm sieve.

The sieved blend was then compressed in a tabletting machine to two-layered chewing gum tablets.

Tabletting was performed by first forming (dosing) 0.4 g sorbitol ((obtained from Roquette; particle size Min 90% > 40μm, Max 5% > 600μm) in a tabletting machine (P/O Weber, Model PW10) and then compressing the material with a pressure of 1000 kg per square centimetre to form a compressed tablet part.

Subsequently, 1.1 g of the sieved **blend of gum base particles and the sorbitol particles** was placed in the tabletting machine on top of the compressed tablet material. Again compression was applied with a pressure of 1000 kg per square centimetre to form a compressed gum part and at the same time completing manufacture of the chewing gum tablet.

The produced tablets were cylindrical in shape, and had a diameter of 16mm and a height of 5,5 mm.

The tabletting step was repeated to prepare a total of 35 chewing gum tablets.

## Comparative Example 1

The procedure of example 1 was repeated, with the following change: rather than making a blend of gum base particles and sorbitol, chewing gum particles were prepared by thoroughly mixing the sorbitol and the gum base components (prior to cooling and grinding) by inclusion of the sorbitol in the Z-blade mixer. Thus compression to form the gum layer was effected on sieved **particles of preformed chewing gum** rather than on a mixture of particles of gum base and sorbitol.

| *Contents and construction of gum part* | *Example 1* | *Comparative Example 1* |
|---|---|---|
| Plasticized rubber | 45% (w/w) | 45% (w/w) |
| Sorbitol | 55% (w/w) | 55% (w/w) |
| Number of different particle types compressed | Two (Gum base particles and sorbitol particles) | One (Preformed chewing gum) |

## *1.2 Sample Analysis*

The chewing gum tablets of Example 1 and Comparative Example 1 were subjected to the following tests in order to examine how the crunchiness of the tablets compared:

- Analytic measurement of hardness of the gum part of the chewing gum tablet;
- Analytic measurement of audible crunchiness of the tablet part of the chewing gum tablet; and
- Sensoric test by a test panel to evaluate crunchiness of the chewing gum tablet as a whole.

### 1.2.1 Analytic measurement of hardness

**Materials:**

- Texture Analyser TA-XT2i from Stable Micro Systems
- Needle probe 4 mm diameter cylinder stainless steel
- Flat Plate with hole (5 mm).

**Principle:**

A probe penetrates 3.5 mm through the gum layer of a compressed chewing gum tablet. The force required to achieve this is recorded (in Newton).

**Measurement of hardness:**

1. Place a compressed gum under the probe, with the gum part facing up.
2. Run a test by moving the probe into the gum part of the sample using the texture analyser (set to a speed of 0,1 mm/sec, data acquisition rate of 200 points/ sec), while monitoring the force required to penetrate through 3.5 mm of the gum part.
3. Repeat 4 more times, each test using a fresh sample– a total of 5 measurements
4. Average the force measurements obtained to arrive at a force value reflecting the hardness of the gum part.

### 1.2.2 Analytic measurement of crunchiness

## Materials:

- Texture Analyser TA-XT2i from Stable Micro Systems
- Cone (70°, diameter=52 mm, height=39 mm)
- Acoustic Envelope Detector (microphone)
- Flat Plate without hole.
- Calibrating module

## Principle:

When the tablet part of a chewing gum is broken or crushed, characteristic sounds are produced due to brittle fracture.

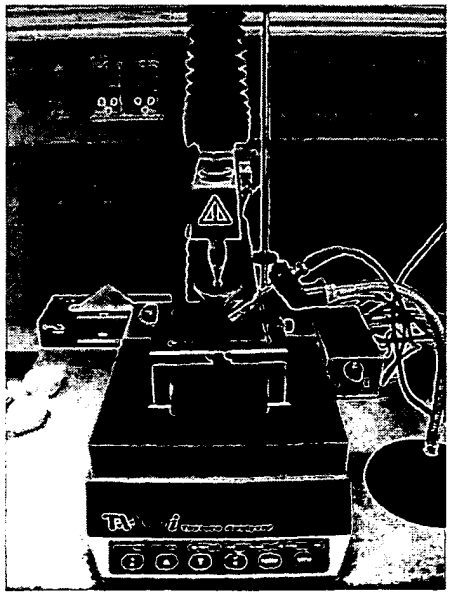
A cone is made to penetrating 3.0 mm through the tablet part of a compressed chewing gum tablet. A microphone (Acoustic Envelope Detector) records and measures noise resulting from the penetration of the cone through the tablet layer (in dB).

The experiment is done in an environment with as little background noise as possible, to compensate for background noise, background noise is measured by conducting the measurement on a foam rubber sample and the background noise dB max measurements are subtracted from the dB max measurements obtained when measuring the chewing gum tablet sample.

## Placement of the microphone and setup of apparatus:

Place the microphone as close as possible to the cone with an angle of 45° to the horizontal plane.

The apparatus is set up as shown in the photograph below:



**Background noise check:**

1. Place the microphone as described above.
2. Place a piece of foam rubber under the cone
3. Run a test by lowering the cone onto the foam, recording and measuring the noise output in terms of the maximum volume (in dB) during lowering of the cone.
4. Repeat 4 more times to obtain a total of 5 measurements

**Measurement of crunchiness:**

5. Place the microphone as described above.
6. Place a compressed chewing gum under the cone, with the tablet part facing towards the cone.
7. Run a test by lowering the cone onto the tablet part of the chewing gum tablet, recording and measuring the noise output in terms of the maximum volume (in dB) during lowering of the cone.
8. Repeat 4 more times to obtain a total of 5 measurements
9. Check that all five data sets are similar; if one or more differ from the remaining data sets by a statistically significant amount, discard that data set due to interference by outside noise sources and repeat the experiment
10. Average the maximum dB values collected in each of the five experiments and subtract the average maximum dB value of the background noise checks to obtain a max dB value for the crunching of the tablet part.

### 1.2.3 Sensorial measurement of crunchiness

Samples of the two different chewing gum tablets were given to a test panel consisting of nine members. These panel members were asked to rate the crunchiness of each chewing gum during the first 30 seconds of chewing on a scale from 0 to 15.

The panel test was carried out in the sensory laboratory of Chewtech, which consists of 21 individual tasting booths according to ISO 8589.

The methodology used is described in ISO 4121-2003.

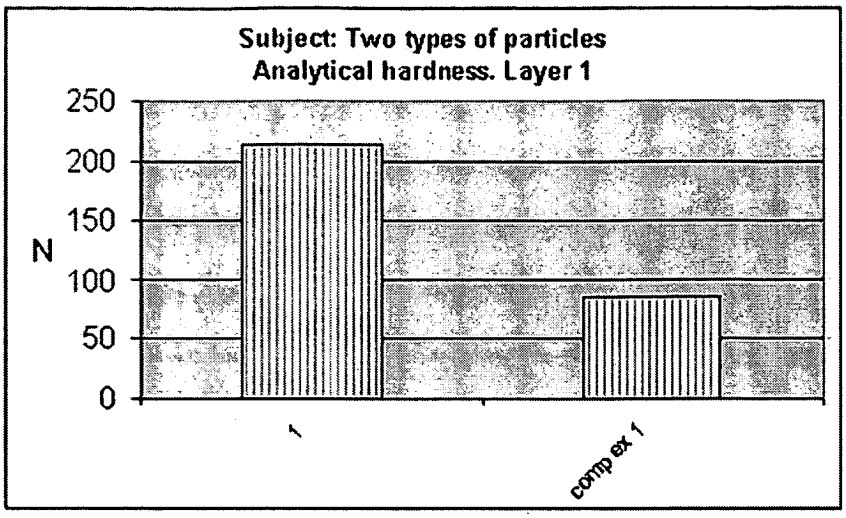
The two products were served at room temperature in small tasteless plastic cups, both samples being randomised coded with a three figure number.

There is a three minutes break between every product there are being tested. The FIZZ (French Bio system) is used to collect and calculate data.

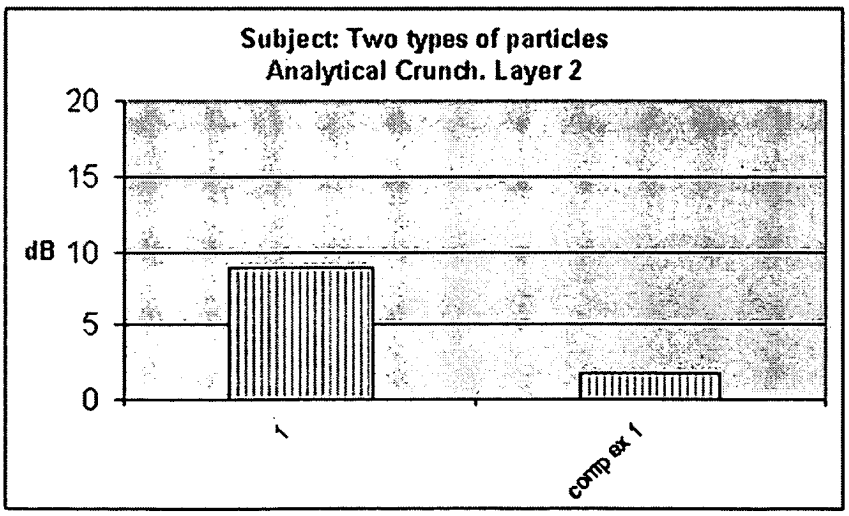
Results were collected as an average of the numerical ratings obtained from the nine panel members.

## *1.3 Results of the tests*

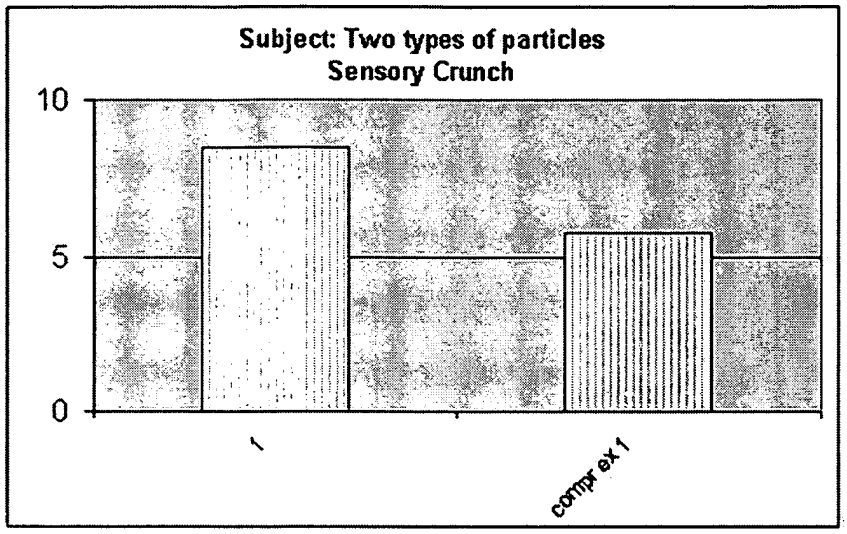
### 1.3.1 Results for analytic measurement of hardness of the gum layer




### 1.3.2. Results for analytic measurement of crunchiness of the tablet layer



### 1.3.3. Results for sensorial evaluation of crunchiness of the chewing gum tablet




| *Experiment* | *Comments from the sensorial judges* |
|---|---|
| *Example 1* | No comments |
| *Comparative Example 1* | No comments |

# Part Two: Crunchiness effect and initial sweetness release effect of plasticized rubber gum base material vs. chicle gum

Aim: To investigate the effects on crunchiness and initial sweetness release of a chewing gum tablet including a tablet part and a gum part (i) when a plasticized rubber gum base material used vs. (ii) when chicle gum is used.

## *2.1 Preparation of samples*

### Example 1

Chewing gum tablets were prepared as outlined for example 1 in section 1.1 above. As explained above, **plasticized rubber gum base particles** were used.

### Comparative Example 2

The procedure of example 1 was repeated, with the following change: rather than making a blend of plasticized rubber gum base particles and sorbitol, a blend of **chicle gum particles** and sorbitol was prepared. The chicle gum particles include paraffin wax, which was added at the blending stage.

| *Contents and construction of gum part* | *Example 1* | *Comparative Example 2* |
|---|---|---|
| Plasticized rubber | 45% (w/w) | - |
| Chicle gum | - | 36% (w/w) |
| Paraffin wax | - | 9 % (w/w) |
| Sorbitol | 55% (w/w) | 55% (w/w) |

## *2.2 Sample Analysis*

The chewing gum tablets of Example 1 and Comparative Example 2 were subjected to the following tests in order to examine how the crunchiness of the tablets compared:

- Analytic measurement of hardness of the gum part of the chewing gum tablet;
- Analytic measurement of audible crunchiness of the tablet part of the chewing gum tablet;
- Sensoric test by a test panel to evaluate crunchiness of the chewing gum tablet as a whole; and
- Analytic measurement of sweetness release.

The above crunchiness tests were conducted in the manner outlined in sections 1.2.1, 1.2.2 and 1.2.3 above. The sweetness release test was conducted as explained below.

### 2.2.1 Analytic measure of sweetness release of the chewing gum tablet using HPLC

## Principle of Release Measurements:

Sweetener content is measure on a chewed and an unchewed gum sample by conducting HPLC measurements of an extract of each gum sample. Sweetener release is measured by subtracting (i) the measured sweetener content of a chewed gum tablet from (ii) the measured sweetener content of an identical unchewed chewing gum tablet. This result is divided by the amount of sweetener measured to have been present in the unchewed gum, and is expressed as a percentage.

## Equipment:

- Chewing machine (mechanical parts manufactured by Hedensted Maskinfabrik A/S; electrical parts and software solutions designed by Kjaergaard; see schematic diagram of chewing machine attached hereto as Annex A)
- Dionex HPLC system consisting of:

| | |
|---|---|
| High pressure pump: | Flow = 0.8 ml/min |
| Auto sampler: | Injection volume = 20 µl |
| Columns: | Shodex Sugar SP-G Guard column<br>Shodex Sugar SP0810 column (Plate number >11.000)<br>A capillary column is inserted right before the pre-column in order to generate a suitable counter-pressure in the system (40-60 bars) |
| Column furnace: | Temperature = 80 °C ± 1°C |
| RI-detector | Temperature = 30 °C ± 1°C |
| UV-detector | Temperature = 30 °C ± 1°C |

Approx. retention times (mins)

| | |
|---|---|
| Sorbitol | 21,5 min |
| Sucrose | 11.2 min |

## Reagents:

- Toluene.
- 0,01 M Disodium hydrogen phosphate buffer (10 mM)
  - 1,78 g $Na_2HPO_4$, $2H_2O$ in 1000 ml demineralised water, pH is adjusted to 3,3 by addition of NaOH.
- ammonium-di-hydrogen-phosphate buffer solution
  - 5,52 g $NH_4H_2PO_4$ is dissolved in 4 litres of demineralised water and the pH is adjusted to 7,4 by addition of NaOH.

## Preparation of Mobile phases and control samples:

**Mobile phases**
Millipore (hereinafter "MP") water degassed in ultrasonic bath for 5 min.

**Standard sample 1**
1,00 g of sorbitol is dissolved in 100 ml MP water.

**Control sample 2**
1,00 g of sucrose is dissolved in 100 ml MP water.

## Quantitative determination of sweetener in chewing gum tablets:

***Analysis of sweetener content of an unchewed chewing gum tablet:***

1. An unchewed chewing gum tablet is extracted with 20 ml. disodium hydrogen phosphate buffer (10 mM) and 15 ml. toluene.
2. The sample is shaken for 2 hours at a frequency of 120 rpm
3. The sample is transferred to a centrifuge tube and centrifuged for 10 minutes at 3000 rpm.
4. Aprox. 2 ml of the aqueous phase is filtered through a 0, 45µm filter and added to an HPLC-vial.
5. HPLC analysis is applied to establish how much sweetener is present in the unchewed tablet.

***Analysis of sweetener content of a chewed chewing gum tablet:***

20 ml of ammonium-di-hydrogen-phosphate buffer solution is introduced in the chewing chamber of the chewing machine.

A chewed sample of the chewing gum tablet is prepared by introducing the chewing gum tablet into the chewing machine with the buffer. 2 horizontal pistons work the chewing gum at a constant frequency (1 Hz, i.e. 60 impacts per minute). A third piston (the tongue) closes the chewing chamber from the top and ensures that the chewing residue stays in the right position before the next horizontal chew. The following conditions are used:

| | |
|---|---|
| Chewing temperature: | 36,5 – 37,4 °C |
| Chewing Frequency: | 60 impacts/min |
| Chewing time: | 1 minute |

1. The chewed samples are removed from the chewing machine, and the buffer is discarded.
2. The chewed gum is extracted with 4,0 ml ammonium-di-hydrogen-phosphate buffer solution and 6,0 ml. toluene
3. The sample is shaken for 2 hours at a frequency of 120 rpm
4. The sample is transferred to a centrifuge tube and centrifuged for 10 minutes at 3000 rpm.
5. Aprox. 2 ml of the aqueous phase is filtered through a 0, 45m filter and added to an HPLC-vial.

6. HPLC analysis is applied to establish how much sweetener is present in the chewed tablet.

The content of sweetener in a chewing gum tablet is:

$$(mg/g\_sample) = \frac{Amount_{mg/ml} \cdot volume_{ml}}{sample_g}$$

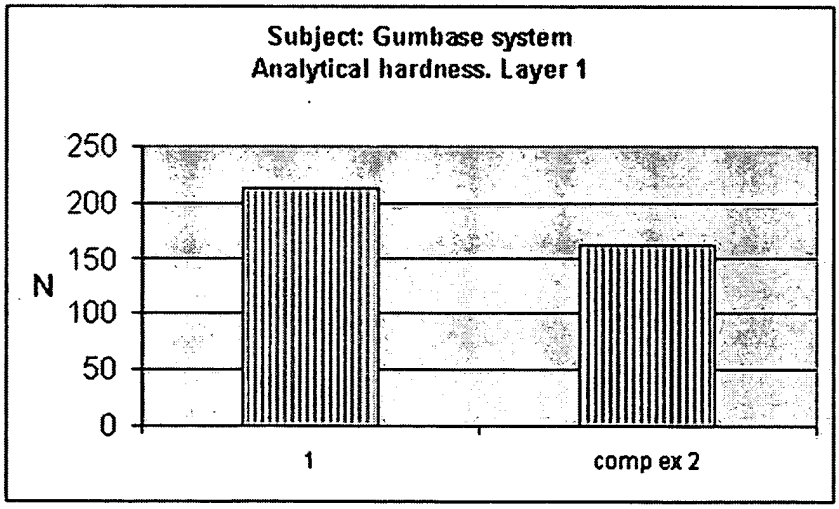
Recovery of the control standards through HPLC must be 95 % - 105 %

Relative standard deviation must be no more than 2.0%.

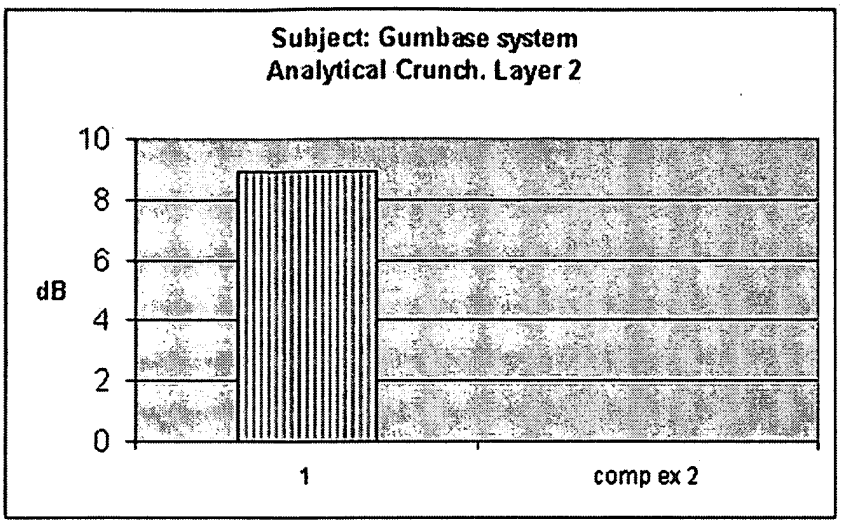
| Parameter | RSD<br>% | Recovery<br>% |
|---|---|---|
| Maximum | 2,0 % | +/- 5 % |

## *2.3 Results of the tests*

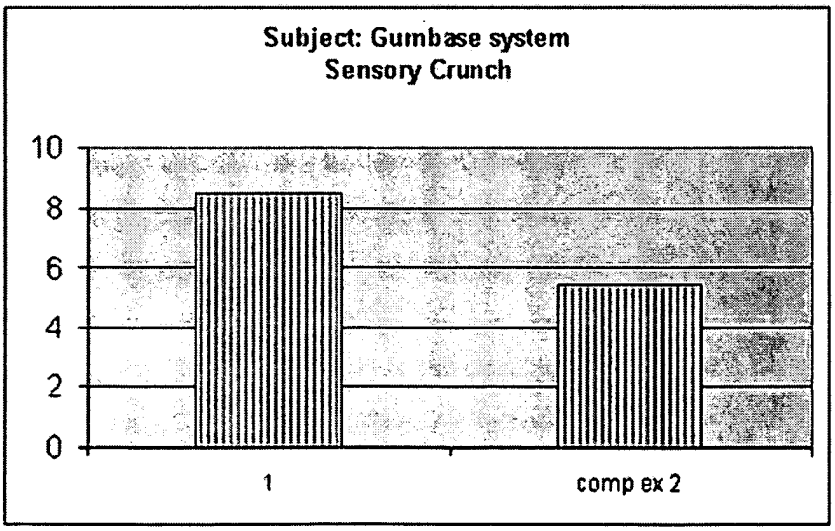
### 2.3.1 Results for analytic measurement of hardness of the gum layer



### 2.3.2 Results for analytic measurement of crunchiness of the tablet layer

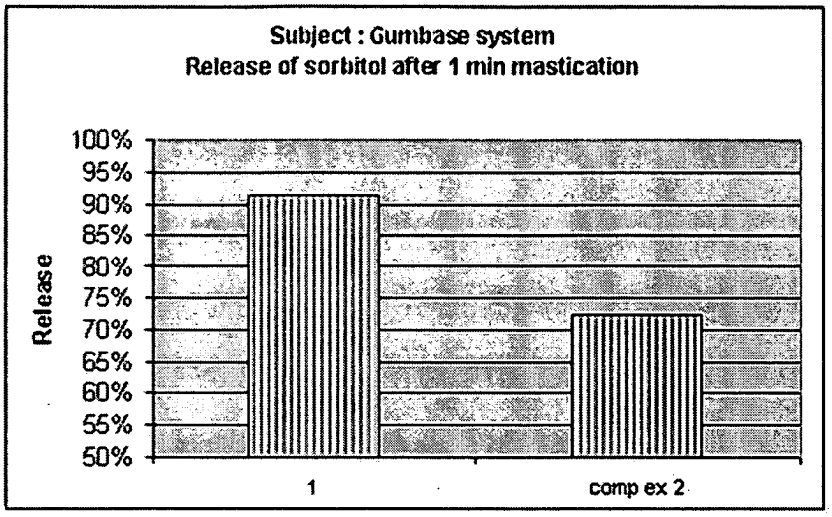



### 2.3.3 Results for sensorial evaluation of crunchiness of the chewing gum tablet




| *Experiment* | *Comments from the sensorial judges* |
|---|---|
| *Example 1* | No comments |
| *Comparative Example 2* | Hard to chew; odd taste; sticks to the teeth. |

## 2.3.4 Results for analytic measurement of sweetness of the chewing gum tablet



# Part Three: Crunchiness effect and initial sweetness release effect of polyol tablet base material vs. sugar tablet base material

Aim: To investigate the effects on crunchiness and initial sweetness release of a chewing gum tablet including a tablet part and a gum part when (i) the tablet base material used is a polyol vs. (ii) the tablet base material is a sugar.

## *3.1 Preparation of samples*

### Reference Example 1

Chewing gum tablets were prepared as outlined for example 1 in section 1.1 above, except that chicle was used in place of the plasticized rubber gum base (as described in comparative example 2 above), and that the chicle and the sorbitol were mixed prior to granulation (as described in comparative example 1 above). As explained above, **sorbitol** was used as the tablet base.

### Comparative Example 3

The procedure of Reference Example 1 was repeated, with the following change: rather than making a blend of chicle particles and sorbitol prior to granulation, a blend of chicle and sucrose was prepared. Thus compression to form the gum layer was effected on a granulated and sieved mixture chicle and **sucrose** rather than on a granulated mixture of chicle and sorbitol.

| *Contents and construction of gum part* | *Reference Example 1* | *Comparative Example 3* |
|---|---|---|
| Chicle gum | 36% (w/w) | 36% (w/w) |
| Paraffin Wax | 9% (w/w) | 9% (w/w) |
| Sorbitol | 55% (w/w) | - |
| Sucrose | - | 55% (w/w) |
| Number of different particle types compressed | One (Preformed chicle and sweetener particles) | One (Preformed chicle and sweetener particles) |

## *3.2 Sample Analysis*

The chewing gum tablets of Reference Example 1 and Comparative Example 2 were subjected to the following tests in order to examine how the crunchiness of the tablets compared:

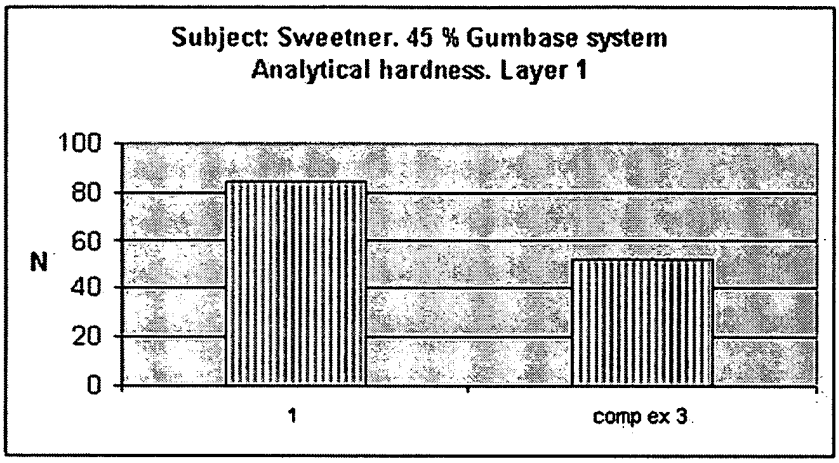
- Analytic measurement of hardness of the gum part of the chewing gum tablet;
- Analytic measurement of audible crunchiness of the tablet part of the chewing gum tablet;

- Sensoric test by a test panel to evaluate crunchiness of the chewing gum tablet as a whole; and
- Analytic measurement of sweetness release.

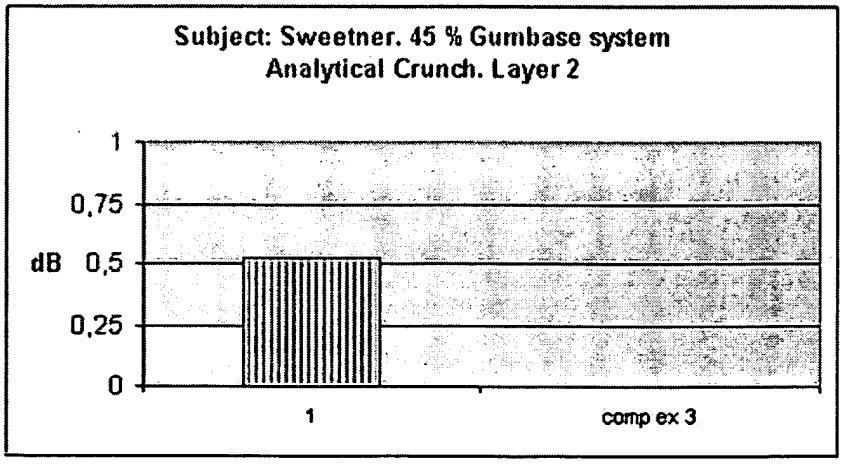
The above tests were conducted in the manner outlined in sections 1.2.1, 1.2.2, 1.2.3 and 2.2.1 respectively.

## *3.3 Results of the tests*

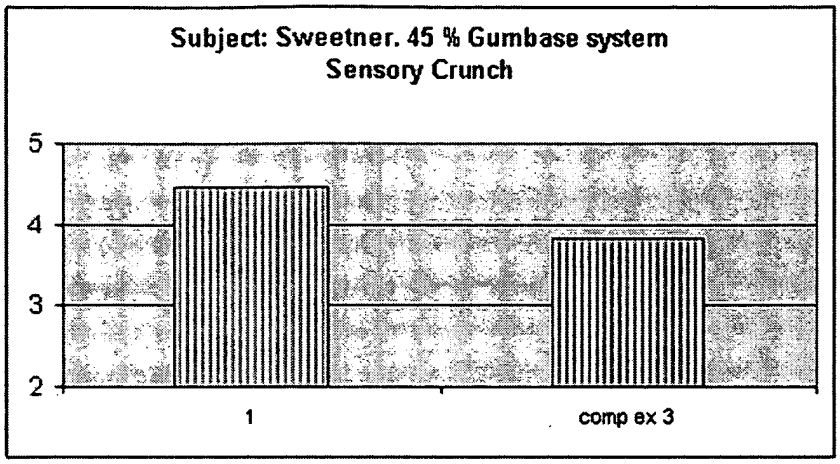
### 3.3.1 Results for analytic measurement of hardness of the gum layer




### 3.3.2 Results for analytic measurement of crunchiness of the tablet layer

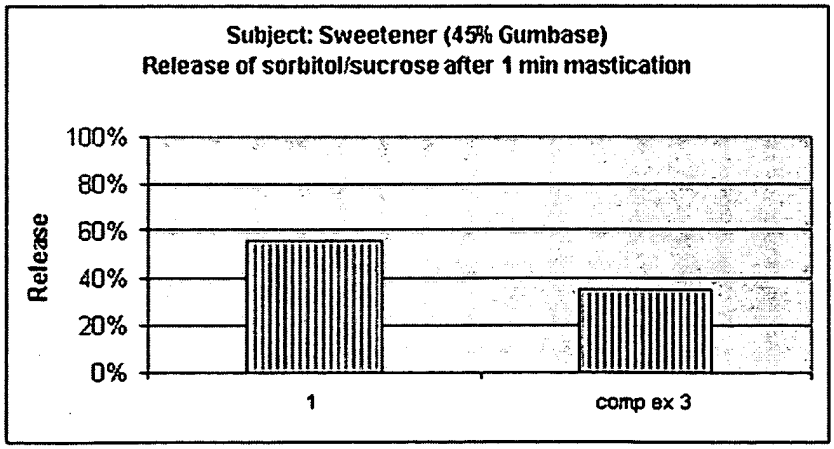


### 3.3.3 Results for sensorial evaluation of crunchiness of the chewing gum tablet




| *Experiment* | *Comments from the sensorial judges* |
|---|---|
| *Reference Example 1* | No Comments. |
| *Comparative Example 3* | Hard and tough initial phase. Sticky in the later phases. |

### 3.3.4 Results for analytic measurement of sweetness of the chewing gum tablet



# Part Four: Crunchiness effect of variation in the gum base content of a chewing gum tablet

Aim: To investigate the effects on crunchiness of a chewing gum tablet including a tablet part and a gum part when the gum base content in the gum part is varied.

## *4.1 Preparation of samples*

### Example 1

Chewing gum tablets were prepared as outlined for example 1 in section 1.1 above. A **45:55** blend of gum base particles and sorbitol was used for the gum part.

### Example 2

The procedure of example 1 was repeated, with the following change: rather than making a 45:55 blend of gum base particles and sorbitol, a **15:85** blend of these ingredients was used.

### Comparative Example 4

The procedure of example 1 was repeated, with the following change: rather than making a 45:55 blend of gum base particles and sorbitol, a **55:45** blend of these ingredients was used.

### Comparative Example 5

The procedure of example 1 was repeated, with the following change: rather than making a 45:55 blend of gum base particles and sorbitol, a **5:95** blend of these ingredients was used.

| *Contents and construction of gum part* | *Comparative Example 5* | *Example 2* | *Example 1* | *Comparative Example 4* |
|---|---|---|---|---|
| Plasticized rubber | 5% (w/w) | 15% (w/w) | 45% (w/w) | 55% (w/w) |
| Sorbitol | 95% (w/w) | 85% (w/w) | 55% (w/w) | 45% (w/w) |

## *4.2 Sample Analysis*

The chewing gum tablets of Example 1 and Comparative Example 3 were subjected to the following tests in order to examine how the crunchiness of the tablets compared:

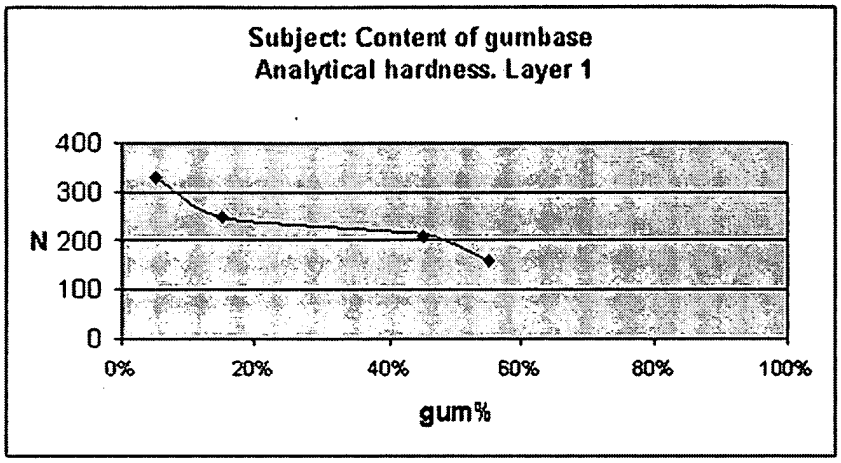
- Analytic measurement of hardness of the gum part of the chewing gum tablet;

- Analytic measurement of audible crunchiness of the tablet part of the chewing gum tablet; and
- Sensoric test by a test panel to evaluate crunchiness of the chewing gum tablet as a whole.

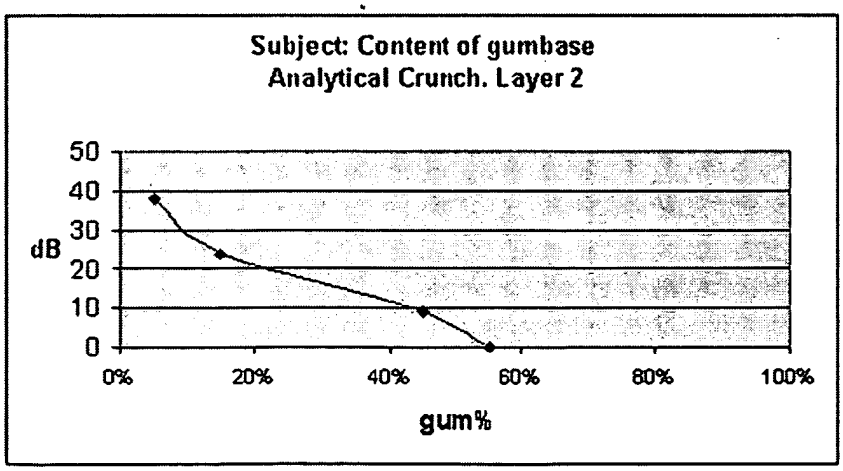
The above tests were conducted in the manner outlined in sections 1.2.1, 1.2.2 and 1.2.3 respectively.

## *4.3 Results of the tests*

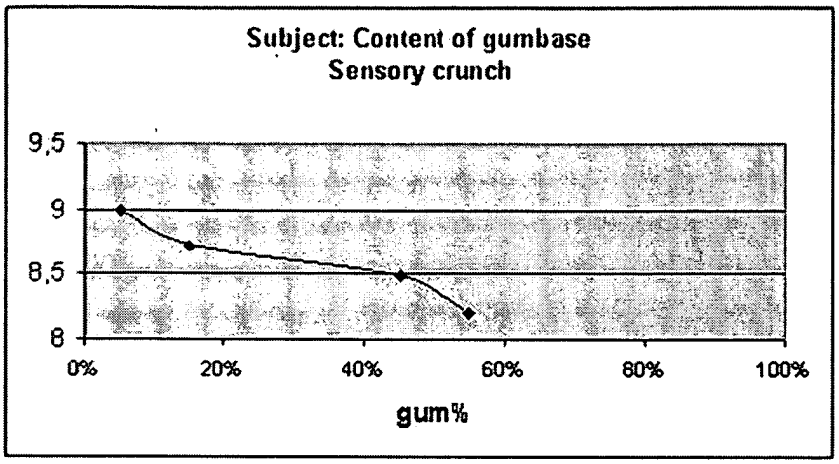
### 4.3.1 Results for analytic measurement of hardness of the gum layer




### 4.3.2 Results for analytic measurement of crunchiness of the tablet layer

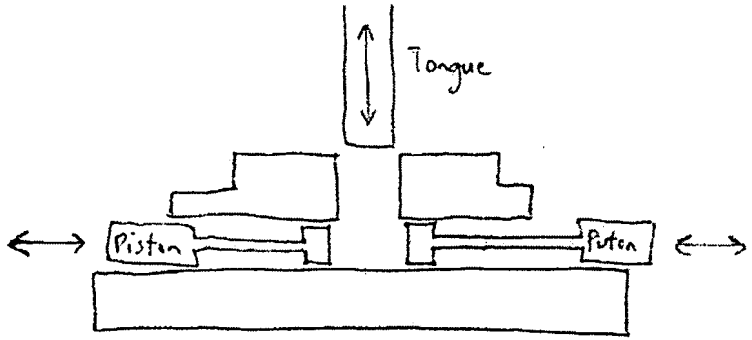


### 4.3.3 Results for sensorial evaluation of crunchiness of the chewing gum tablet




| *Experiment* | *Comments from the sensorial judges* |
|---|---|
| Example 1 | No comments |
| Example 2 | No comments |
| Comparative Example 4 | No comments |
| Comparative Example 5 | Very high hardness. Small gum volume. |

ANNEX A: The chewing machine (princip) "one chamber"




Illustrating the principle of the machine developed by Fertin Pharma for measuring release of active substances under standardised conditions.

The machine is accepted in the European Pharmacopoeia as the standard method for measuring release of active substances from chewing gum.